Application of a Micro-PCR Instrument for Fast, Reproducible and Field-Portable Detection of Clostridium perfringens.

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The α-toxin of Clostridium perfringens is responsible for such diverse human diseases as food poisoning, necrotic enteritis of infants and gas gangrene. PCR has emerged as a sensitive and specific method to detect C. perfringens in clinical and soil or sewage samples but its application has been limited to the laboratory setting supporting full scale thermocycler units. Here we compare the performance of a micro-PCR unit developed at Lawrence Livermore National Laboratory to that of the full scale themocycler in terms of reproducibility and amount of amplicon produced in the detection of C. perfringens. The α -toxin is produced by all types of C. perfringens (types A,B, C, D, and E) and detection of this gene is a powerful clinical and environmental diagnostic tool. Therefore it was chosen as the target for PCR. Five primer pairs which are specific to the α-toxin of C. perfringens and one primer pair representing a conserved region of the 16S rRNA gene in bacteria were used in amplification reactions targeting C. perfringens. The micro-PCR unit produced amplicons of the same size and in all but one case, the same intensity as amplicons from the full-size thermocycler. Typical cycle times were 1.6h for the full-scale thermocycler but less than 20 minutes for the micro-PCR unit due to its faster thermotransfer capabilities. Most significantly, the micro-PCR device can be brought to the field, providing immediate detection of pathogens and obviating risks associated with sample transport. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract No. W-7405-ENG-48.